Product Insert

BIO-XA-CT™ DNA Polymerases

Research Use Only

Product:
BIO-X-ACT™ Short DNA Polymerase
BIO-X-ACT™ Long DNA Polymerase

Catalogue No.:
BIO-X-ACT Long
BIO-21049 250 units
BIO-21050 500 units

BIO-X-ACT Short
BIO-21064 250 units
BIO-21065 500 units

Description:
BIO-X-ACT™ DNA polymerase is a high-performance proprietary complex of enzymes specifically designed for difficult/problematic applications requiring high processivity with high-fidelity. Two versions of BIO-X-ACT are available for short and long fragments respectively.

BIO-X-ACT™-Short DNA Polymerase is recommended for short Genomic DNA fragments of up to 2Kb, or up to 5Kb on Lambda DNA. With Lambda DNA as template, the best performance is achieved within the 100bp to 3Kb range.

BIO-X-ACT™-Long DNA Polymerase is recommended for longer Genomic DNA fragments of between 2-20Kb, or up to 30Kb Lambda DNA fragments. With Lambda DNA as template, the best performance is achieved in the 2-20Kb range. BIO-X-ACT-Long is our original widely used BIO-X-ACT formulation**.**

Concentration:
4u/µl

10x reaction buffer:
OptiBuffer™

Specificity Enhancer:
5x Hi-Spec Additive is a specificity enhancer.
If necessary, re-dissolve Hi-Spec by heating to 70ºC and vortexing.

Separate MgCl2 solution:
50mM $MgCl_2$

Storage Conditions:
BIO-X-ACT™ DNA Polymerase can be stored at -20°C, in a constant temperature freezer for 12 months. BIO-X-ACT™ will remain stable if stored as specified.

Shipping Conditions:
At +4ºC or -20ºC.

Unit definition;
One unit is defined as the amount that incorporates 10nmoles of dNTP's into acid-precipitable form in 30 minutes at 72°C.

Storage buffer:
20mM Tris-HCl, pH 7.5, 100mM NaCl, 0.1mM EDTA, 2mM DTT, 50% Glycerol, and 0.1% Tween-20.

Associated activities:
Endonuclease and exonuclease activities were not detectable after 4 hours of incubation of 1µg of pBR322 plasmid DNA and 0.5µg Hind III-digested lambda DNA at 72°C in the presence of 20 units of BIO-X-ACT.

Note: This product is supplied for use in primer extension reactions. Purchase of this product does not convey a licence to perform any patented process.

Associated products:

Product Name:	Pack Size	Cat No:
dNTP Set	4 x 25µmol	BIO-39025
dNTP Mix:		
100mM total	1 x 500µl	BIO-39028
40mM total	1 x 500µl	BIO-39043
2x Poly-Mate Additive	2 x 1.2ml	BIO-37041
Hyper Ladder I	200 lanes	BIO-33025
Agarose	500g	BIO-41025

This product contains a declaration of analysis at the time of manufacture

See Overleaf for Features and Applications

Features and applications:

Long-Region Applications: Optimal composition of different enzymatic activities enables **BIO-X-ACT™ Long** to span the primer extension over long regions and demonstrate high processivity by reducing premature strand termination and template degradation. Using long primers at elevated Mg++ concentrations, >30kb or 20kb products can be achieved from lambda templates or genomic DNA, respectively.

BIO-X-ACT™ Short is a newer member of the BIO-X-ACT™ family of polymerases, and is designed specifically for short-region applications of <2kb.

The main characteristics of **BIO-X-ACT™ Long** and **BIO-X-ACT™ -Short** remain the same.

•**Difficult Templates:** BIO-X-ACT™ provides high performance and specificity, even with 'dirty' DNA or difficult templates with an unfavorable nucleotide composition. In contrast to standard 3'-5' proof-reading polymerases, BIO-X-ACT™ can be used in combination with degenerate or non-perfect matching primers.

• **A' Overhang:** BIO-X-ACT™ is recommended for direct gene cloning without the need to verify the sequence prior to expression. BIO-X-ACT™ leaves an A' overhang such that the primer extension product is suitable for effective integration into TA cloning vectors, even from difficult templates.

•**High Fidelity:** BIO-X-ACT™ is a mix of polymerases that possesses a 5'-3' DNA polymerase activity and 3'-5' proof-reading activity which reduces misincorporations during primer extension. This combination of properties provides a >17 fold higher fidelity than *Taq*. In contrast with other proof-reading enzymes, BIO-X-ACT™ does not degrade primers.

•**High Specificity:** BIO-X-ACT™ is supplied with a vial of a unique specificity enhancer. **5x Hi-Spec additive** helps to prevent the formation of false background bands and smearing, especially on difficult templates. Hi-Spec Additive should be used at 1.0-2.0x final concentration – the optimal amount required should be determined for each individual experiment. Hi-Spec Additive may also alter the ideal annealing temperature for primers – some optimisation may be required.

Specificity and performance of the BIO-X-ACT™ Polymerases can be increased more with the use of **2x Poly-Mate** (not supplied) which is the improved version of **Hi-Spec additive** designed for cases of GC-, or AT-rich, "dirty" templates, or sequences with difficult melting profiles.

Reaction Conditions

For a 50µl Reaction

10x OptiBuffer (provided)	5µl
$MgCl_2$, 50mM Solution (provided)	2-8 µl
100mM dNTP Mix (see below)	0.5-1.0 µl
Template and Primers	as required
Enzyme	0.5-2µl
Water (ddH_2O)	up to 50µl

Bioline 100mM dNTP rmix is available as a separate product (catalogue number BIO-39028)

Denature: 94-96°C
Elongate: 68°C (40-60 Seconds per 1Kb)

This data is intended for use as a guide only, conditions will vary from reaction to reaction and may need optimisation.

Troubleshooting

Observation	Recommended solution(s)
No or low yield of extended product	For Difficult templates (AT and GC rich). Try 2x Poly-Mate (BIO-37041) to lower the melting profile and improve performance.
	Enzyme Concentration too low – increase the amount enzyme in 0.5U increments.
	Magnesium Concentration too low – increase concentration in 0.25mM increments with a starting concentration of 1.75mM.
	Primer Concentration not optimised. Titrate primer concentration (0.3-1µM); ensuring that both primers have the same concentration.
Multiple bands	Primer Annealing temperature too low. Increase annealing temperature. Primer annealing should be at least 5°C below the calculated Tm of primers.
	Prepare master mixes on ice or perform a hot-start step.
	For problems with low specificity. Try Hi-Spec Additive or 2x Poly-Mate (not supplied) to improve specificity.
Smearing or artefacts	Template concentration too high. Prepare serial dilutions of template.
	Too Many Cycles. Reduce the cycle number by 3-5 to remove non-specific bands.
	Enzyme Concentration too high – decrease the amount of enzyme in 0.5U increments.
	Extension Time too Long. Reduce Extension time in 0.5-1 minute increments.